Unconventional Myosins

627-Pos Board B427
Three Dimensional Orientation Measurements of Single Fluorescent Molecules by Newly Developed Polarized Fluorescence Microscopy
Masashi Ohmachi, Yasunori Komori, Atsuko Iwane, Fumihiko Fujii, Toshio Yanagida.
Single molecule fluorescence techniques are increasingly important to observe the dynamic properties of single molecules. One such important dynamic property is the single molecule's orientation. In order to observe three dimensional motions of proteins in solution, it is necessary to measure three dimensional orientations of proteins. We developed new microscopy for determining three dimensional orientations based on the principal of polarization analysis proposed by Fourkas. This method requires only that one collect fluorescence counts from a single molecule at three different polarizations followed by a simple mathematical calculation to yield the three dimensional orientations. In this method, the relatively small numbers of photons are sufficient for a reliable orientation measurement and this should decrease the time scale needed to determine the orientation of any given fluorophore. Here, we demonstrate axial rotation of actin filaments sliding over myosin molecules fixed on a glass surface by polarization measurement of individual rhodamine phalloidin fluorophores sparsely bound to filaments. This new microscopy will be available for investigating the wide range of dynamic processes through single molecule orientation dynamics in various biophysical studies.

628-Pos Board B428
Conformational Dynamics of Nucleotide-Free Myo1b
Henry Shuman, Joseph M. Laakso, **E. Michael Ostap**.
Myo1b is the widely expressed myosin-I isoform with kinetics that are highly sensitive to tension (Laakso et al. 2008. Science. 321:133-6). To better understand the structural transitions that accompany the myo1b power stroke, we analyzed the displacement of actin filaments by single myo1b molecules in a 3-bead trap assay. Under conditions where we predict myo1b to be nucleotide-free, we observe ''flickering'' of the position of myo1b-bound actin between two distinct states. We are able to measure the rate of transition between these states, and we have found the rates to be force sensitive. Interestingly, previous biochemical kinetic measurements have shown that actin-bound myo1b (in the absence of nucleotide) is in equilibrium between a state that does not bind nucleotide (AM) and a state can bind nucleotide (AM'). The rate of the transition from the AM to AM' state is similar to the rate of ADP release, and it was proposed (Geeves et al. 2000. J. Biol. Chem. 275:21624-30) that this conformational transition is similar to the conformational change that accompanies ADP release. Our observed flickering rates are similar to the AM to AM' transitions rates. Because we found ADP release to be the force-sensitive transition, it is interesting to consider the role of this nucleotide-free transition in myo1b tension-sensing.

629-Pos Board B429
A Hearing-Loss Associated Myo1c Mutation (r156w) Decreases the Myosin Duty Ratio and Force Sensitivity
Michael J. Greenberg, Tianming Lin, Jeffrey R. Moore, E. Michael Ostap.
Myo1c is a member of the myosin superfamily that has been proposed to function as the adaptation motor in vestibular and auditory hair cells. A recent study identified a myo1c point mutation (R156W) in a person with bilateral sensorineural hearing loss. This mutation is located at the start of the highly conserved switch-1 region, which is a crucial element for the binding of nucleotide. We characterized the key steps on the ATPase pathway at 37°C using recombinant wild-type (myo1c^{3IQ}) and mutant myo1c (R156W-myo1c^{3IQ}) constructs that consist of the motor domain and three IQ motifs. The R156W mutation only moderately affects the rates of ATP binding, ATP-induced actomyosin dissociation, and ADP release. The actin-activated ATPase rate of the mutant is inhibited > 4-fold, which is likely due to a decrease in the rate of phosphate release. The rate of actin gliding, as measured by the in vitro motility assay, is unaffected by the mutation at high myosin surface densities, but actin gliding is substantially reduced at low surface densities of R156W-myo1c^{3IQ}. We used a frictional-loading assay to measure the affect of resisting forces on the rate of actin gliding and found that R156W-myo1c^{3IQ} is less force sensitive than myo1c^{3IQ}. Taken together, these results indicate that myo1c with the R156W mutation has a lower duty ratio than the wild-type protein, and it has motile properties that are less sensitive to resisting forces.

630-Pos Board B430
Studies of the Force-Dependent Motor Activity of Myosin I
Michael Jared Green, Jesse Sant, Ian McGahan, Alex R. Dunn, **David M. Altman**.
Class I myosins are implicated in membrane dynamics, cell structure, and mechanical signal transduction. Broadly speaking, these various roles can be divided into two categories, 1) trafficking function and 2) structural function. It is not clear how myosin I can achieve such disparate functions. One possibility is that the motor's function is regulated by interactions with other proteins and organelles in its cellular environment, a form of regulation that is observed with a class VI myosin. An alternate hypothesis is that the motor changes its activity in response to external forces. External forces selectively perturb mechanical transitions in a motor's mechanochemical cycle, and such perturbations can modulate the motor's kinetics and alter its function. This hypothesis is supported by optical trap studies of a myosin 1b construct, which demonstrated that myosin I is exquisitely sensitive to forces opposing its motion (Laasko et al., Science, 321 (5885):133-136). We tested this model by conducting single-molecule studies of the motor activity of Acanthamoeba myosin Ic (AM1C). Specifically, we used an optical trap assay that allows us to apply forces in various directions relative to the motor's motion along an actin filament.

631-Pos Board B431
Direct Observation of the Myosin Power Stroke and its Reversal
Claudia Veigel, Christopher Batters, Christopher P. Toseland, James R. Sellers.
Cell locomotion and division, organelle trafficking or signal amplification in hearing are complex forms of cellular motility that require strong coordination of the myosin motors involved. The most basic mechanism of coordination is the direct mechanical interaction of individual myosin motor heads, leading to modification and regulation of their mechano-chemical cycles. We have used an optical tweezers-based assay to study the mechanical response of a single myosin-V motor head to a range of loads. We found this response to be non-linear, including reversibility of the force- generating conformational change (power stroke) of single myosin-V motor heads at intermediate forces. By applying load to the head shortly after binding to actin, we found that at 2-4 pN the power stroke could be reversed and the head fluctuated between an actin-bound pre- and a post-power stroke conformation. Load-dependent mechanical instability might be critical to coordinate the heads of processive, dimeric myosin-V. Non-linear response to load leading to coordination or oscillations amongst motors might be relevant for many cellular functions, including those that involve other members of the myosin superfamily.
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Allosteric Tuning of Myosin 5a Motor Activity
Nikolett T. Nagy, Mihaly Kovacs.
Myosin 5a is a processive vesicle transporter capable of taking multiple steps without detachment from actin. Its translocation activity, which powers cargo transport to micrometer distances, requires a range of biochemical adaptations. In this study we engineered the activity of myosin 5a by introducing mutations into two key regions of the motor domain. G227 is located at the entrance of the nucleotide binding pocket. This position is occupied by Gly only in highly processive vertebrate myosin 5a and 5b isoforms, whereas all other myosin 5 isoforms and myosins from other classes possess larger amino acids at this position. Our results show that the G227A mutation in myosin 5a causes a change in the rate-limiting step, which is ADP release in the wild type enzyme. In the mutant, a structural change taking place after ATP hydrolysis and before ADP release becomes rate limiting. The ADP release rate constant is much higher than that of the steady-state ATPase activity. Surprisingly, however, the mutant displays even higher steady-state actin attachment ratio than wild-type myosin 5a. The other region mutated in this study is the interface between the N-terminal and converter subdomains. In myosin 2, a repulsive interaction in this interface (K84-R704 in Dictyostelium myosin 2) exerts a kinetic tuning effect during the hydrolytic cycle, as determined in earlier studies. In wild-type myosin 5a this repulsive interaction is absent as the positive charge is missing at the position homologous to K84 (I67 in mouse myosin 5a). The introduction of a repulsive interaction by the I67K replacement results in a rate-limiting structural transition preceding the ATP-induced dissociation of myosin heads from actin. Thus, both studied mutations cause marked changes in the steady-state distribution of myosin structural states, which in turn alter the mechanochemical output of myosin 5a.